Chromatin structure of *Drosophila melanogaster* ribosomal genes

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The chromatin structure of ribosomal genes of *D. melanogaster* has been studied by crosslinking proteins to DNA. We found that a number of histone contacts with DNA through histidine in the approximately 1 kb-long region surrounding the transcription initiation site, coding regions and the region of 240 bp-long repeats from the intergenic spacers (*Alu*-repeats) were weakened as compared to the inactive chromatin of the type II insertion. A protein with the molecular mass of 50 kDa (p50), associated with all DNA sequences analysed, has been discovered. Another protein with molecular mass of about 70 kDa (p70) has been found to be specific only for the *Alu*-repeats.

DNA-protein crosslinking; Chromatin structure; Ribosomal gene; *D. melanogaster*

1. INTRODUCTION

The *D. melanogaster* haploid genome contains about 250 ribosomal genes, organized in tandem repeats. More than half of the genes contain insertions at about 7 kb downstream from the transcription initiation site [1,2]. Genes with insertions are not transcribed and the mechanism of the transcription inactivation caused by the insertions remains rather obscure [2,3].

An interesting feature of a number of eukaryotic ribosomal genes is the homology of the nontranscribed spacer with the region of the beginning of transcription [4]. For example, the region of transcription initiation in *D. melanogaster* is preceded by 6–8 tandem repeats, excized by *Alu*I restriction endonuclease, wherein 50 bp copy to the region situated from −24 to +27 with respect to the point of transcription initiation [5–7]. Using the 'protein image' hybridization assay based on different crosslinking method [8], some striking differences in the chromatin organization of various regions of *D. melanogaster* have been shown.

2. EXPERIMENTAL

Growth of *D. melanogaster* tissue-culture cells was described earlier [9]. Cells in medium (about 5–10 × 10^6 cells/ml) were transferred to a thermostatic (0°C) vessel and irradiated for 12 min. The cell suspension was less than 0.5 mm in depth. The source of UV-light was an inverted transilluminator (UV-products, USA) without filter, placed 10 cm above the cell suspension. Nuclei were isolated from irradiated cells as described [10], then lysed in 0.15 M NaCl, 10 mM Tris-HCl (pH 8.0), 1 mM EDTA, 0.1% NP-40, 2% lauryl sarcosinate, sonicated for 5 s at 44 kHz and the solution was centrifuged at 6000 × *g* to remove insolubles. To remove uncrosslinked proteins, the supernatant was loaded onto CsCl gradients [10]. Centrifugation was carried out at 16°C for 48 h at200 000 × *g*. The protein-containing top layer was discarded and the DNA-containing fractions were collected and dialysed against 0.25% NP-40, 10 mM Tris-HCl (pH 8.0). $MgCl_2$ was added to 5 mM and DNA and DNA-protein complexes were treated with restrictase *Alu*I (1 unit of enzyme per 1 μg of DNA). Digestion was done at 37°C for 16 h in the presence of 0.5 mM of diisopropylfluorophosphate. To remove most of the uncrosslinked DNA, the phenol enrichment procedure was used [8]. Two-dimensional electrophoresis of the protein-DNA complexes was performed as described in [8] to obtain 'protein images'. The DNA fragments were transferred onto a Hybond nylon membrane (Amersham). The conditions for DNA labeling, hybridization and washing of the filter were all as in [11].

The conditions of crosslinking histones to partially depurinated DNA were described earlier [8].

The conditions of one-dimensional scanning of the autoradiographs of the blots were as described [8].

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3. RESULTS

The histone composition of different chromatin regions in *D. melanogaster* ribosomal repeat was studied by the 'protein image' hybridization technique [8] combined with DNA-protein crosslinking induced by dimethylsulfate. In our protocol, when histones are crosslinked to DNA via histidines which enables us to analyse the contacts of the 'globular' domains of histones with DNA, that play an important role in the folding of nucleosomal DNA and the packaging of nucleosomal fibril [12,13]. Uncrosslinked DNA, DNA bound to core histones, and DNA bound to histone H1 were separated into 3 distinct diagonals by two-dimensional diagonal gel electrophoresis. The intensities of diagonals on blot autoradiographs were quantitatively compared using one-dimensional densitometric scanning horizontally across the diagonals. In this way we obtained quantitative estimates of

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histone crosslinking to DNA through histidines in different regions of the ribosomal repeat. These data are presented in Fig. 1. The region of type II insertion is the richest in histones (Fig. 1, d), while its level of transcription is negligible: no more than 1–2 copies of transcripts per cell [14]. So the insertion can be considered as repressed, inactive chromatin. Judging from Fig. 1 the content of core histones and histone H1 contacts with DNA via histidines increases in the following order: about 1 kb around the transcription origin (Fig. 1,a)< the promoter-like 240 bp repeats within the spacers (Fig. 1,b)< the coding region 5′ and 3′ to the site of insertion (Fig. 1,c,e)< inactive type II insertions (Fig. 1,d).

The 'protein image' assay in combination with UV-light crosslinking method was also used to analyse the protein content in certain regions of the ribosomal repeat. DNA and DNA-protein complexes were isolated from the irradiated *D. melanogaster* cells, treated with restriction endonuclease *AluI* and subjected to two-dimensional gel electrophoresis. After electrotransfer and hybridization there appears a spot (or a number of spots) originated by the non-random degradation of DNA (Fig. 2).

While hybridized with the probe presenting *Alu*-repeat, spots corresponding to 'protein images' of histone HI and two non-histone proteins with molecular mass of about 50 kDa and 70 kDa were revealed. The molecular mass of the proteins was found from model calibration experiments on chemical crosslinking of individual proteins of known molecular mass to DNA.

The same filter was hybridized in succession with probes representing the ribosomal type I and II non-transcribed insertions and coding regions. We found no essential differences in the efficiency of histone HI crosslinking to the sequences of the insertions. The extent of the HI crosslinking in the coding regions and the region of the *Alu*-repeat was weakened about two-fold when compared with the chromatin of the insertions. It was also found that p50 was specific to all sequences analysed, although we noted that it did not crosslink to every fragment generated by *AluI*.

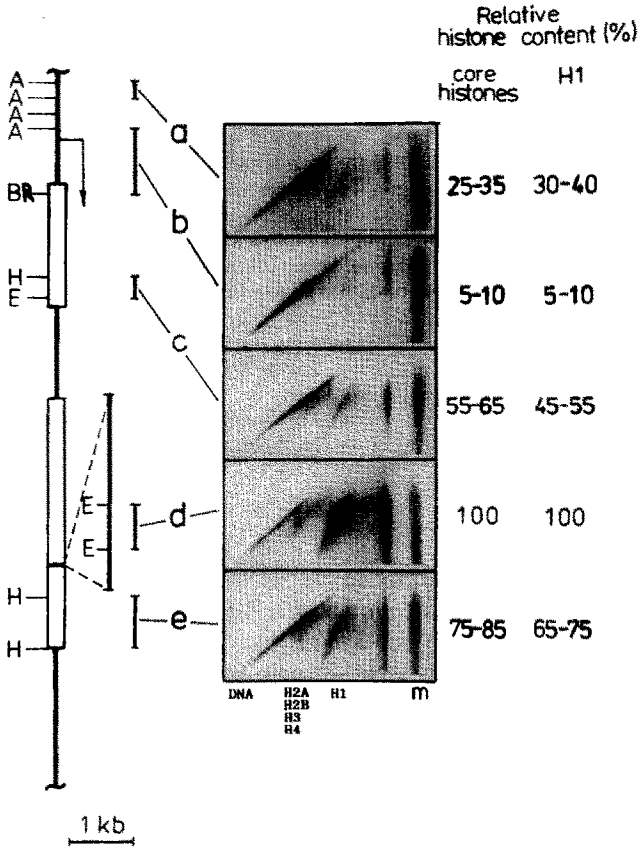

Fig. 1. 'Protein image' hybridizations of different regions in ribosomal genes of *D. melanogaster* (dimethylsulfate-induced crosslinking). A map of the *D. melanogaster* ribosomal repeat is shown to the left, also shown here are the hybridization probes. Restriction sites used for obtaining hybridization probes are designated by capital letters: A, *Alu*I; BR, *Bsp*R, H, *Hin*dIII, E, *Eco*RI. The picture shows autoradiographs obtained after hybridization of the above-mentioned probes to the same filter. The efficiency of histone crosslinking in different regions was estimated relative to the efficiency of crosslinking to the sequences of the type II insertion.

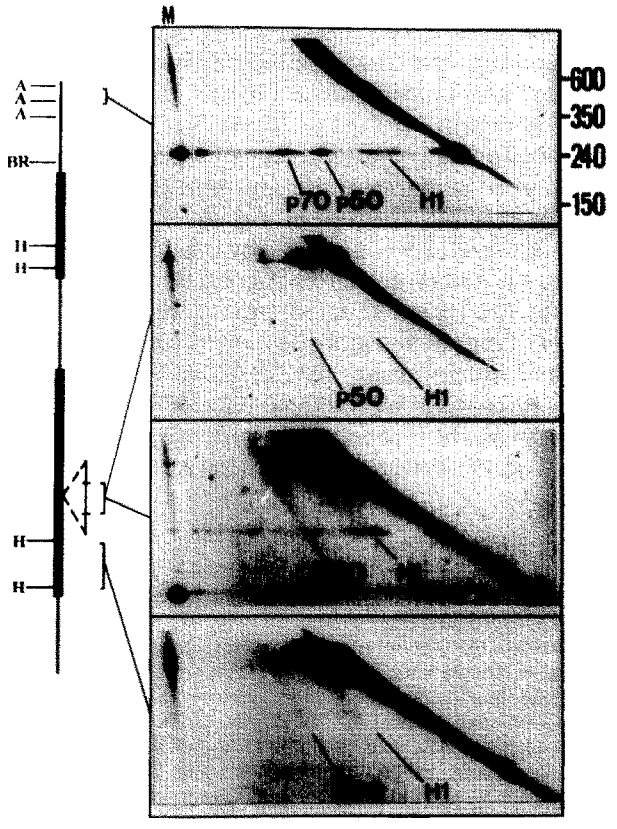

Fig. 2. 'Protein image' hybridization of different regions in ribosomal genes of *D. melanogaster* (UV-crosslinking). Autoradiographs obtained after hybridization with *Alu*-repeat probe, probes represent the type I insertion, the type I insertion and the coding region, correspondingly (from top to bottom).

We were unable to detect DNA-RNA-polymerase I contacts within the region analysed, because of a rather low efficiency of RNA-polymerase crosslinking; the same could be true in case of some other proteins.

4. DISCUSSION

The combination of different protein-DNA crosslinking methods is a convenient method for studying chromatin structure. Earlier we showed that histone-DNA contacts were rearranged during transcription activation of the *hsp-70* gene of *D. melanogaster* [8]. The same approach used in this work has revealed some essential changes in the spectrum of histone-DNA contacts registered by crosslinking methods. The interpretation of the obtained results was rather difficult considering the fact that among 250 ribosomal genes of haploid *D. melanogaster* genome only about 50 are transcribed in embryonic tissue culture cells [15]. But it is known that non-transcribed genes (with insertions) are less sensitive to DNase I than genes without insertions [16]. This fact shows the differences between them in the chromatin fiber organization. So interpreting the data above we can operate only with an averaged picture of the histone contacts, whereas the loss of the contacts in the coding region of the active genes is actually more substantial.

We have found that the region around transcription initiation site (-150 – $+810$ relative to the initiation point) contains only about 10% of the core histones and histone H1 (Fig. 1,b). The active conformation exists here despite the fact that only 20% of *Drosophila* ribosomal genes are transcribed. It should be mentioned here that the RNA polymerase II heat shock promoter (*hsp 70*) is also free of histones irrespective of its transcriptional state (active or inactive) [8,17]. Moving along the transcriptional unit, the histone content increases (Fig. 1,c) and is greatest in the region of the inactive type II insertion (Fig. 1,d). The latter region is especially rich in histone H1, probably due to cooperative binding of H1 molecules to the compacted nucleohistone fiber. At the end of the transcription unit (Fig. 1,e), the amount of histones declines by about one fourth, corresponding to the portion of the genes that may be transcribed along their entire length.

From our data all initiation sites, including those of genes with insertions, exist in an active conformation and are free of histones. Insertions contain a full complement of histones and are therefore in highly condensed chromatin. It could be this compacted conformation of chromatin that hinders the progress of RNA polymerase along the transcription unit and suppresses transcription. On the other hand, fully transcribed ribosomal genes might be thought of as being completely free of histones throughout their length, as demonstrated earlier for the *Xenopus* ribosomal genes [18].

Our hypothesis that the specific features of chromatin organization within insertions are responsible for the suppression of transcription is further corroborated by the results of Dawid and Rebbert [19]. They showed that intercalators drastically increased transcription of genes containing type I insertion. Intercalators are known to cause decondensation of chromatin, unfolding of nucleosomes, and partial removal of core histones. It seems that a decrease in the extent of chromatin condensation, such as in the transition from 30 nm- to 10 nm-fibers, considerably facilitates transcription.

The *Alu*-repeat region is also in active conformation. But one must take into consideration the effect of the extraordinary long nucleosomal repeat demonstrated in this region of *Drosophila* chromatin (240 bp instead of 185 bp) (unpublished observations). As to the binding in the *Alu*-repeat of two nonhistone proteins p50 and p70, one can assume them (or one of them) to stabilize the abnormal chromatin organization in this region. As regards the functional role of these proteins, we think that the DNA-protein binding fixes the position of the histone octamer on DNA, thereby opening (or closing) specific sequences recognized by transcription factors and (or) RNA-polymerase.

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